REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. Recipient's Accession No.
4. Title and Subtitle: MELATONIN ACTION ON THE CIRCADIAN PACEMAKER IN SIBERIAN HAMSTERS			5. Report Date: 10/31/92 6.
7. Author(s): Janet M. Darrow, PhD			8. Performing Organization Rept. No.: #12.800
9. Performing Organization Name and Address: Northeastern University, Office of Sponsored Projects, 360 Huntington Avenue, Boston, MA 02115			10. Project/Task/Work Unit No.: PR/TA: 2312/A3 11. Contract(C) or Grant(G) No.: (C) (G) AFOSR-91-0393
12. Sponsoring Organization Name and Address: Life Sciences Directorate, Air Force Office of Scientific Research, Bolling Air Force Base, D.C. 20332-6448, Dr Genevieve Haddad NL			13. Type of Report & Period Covered: Final Technical Report 9/1/91 - 8/31/92 14.
15. Supplementary Notes			

16. Abstract (Limit: 200 words)

This research investigates the effect of the hormone melatonin on the circadian clock of mammals, by examining daily locomotor activity rhythms in melatonin-infused Siberian hamsters, under a variety of environmental lighting conditions. Under conditions of constant darkness, daily melatonin infusions synchronized the hamster activity/rest rhythm. In constant light, melatonin also acted as a weak entraining agent and prevented the internal desynchronization which occurs in Siberian hamsters and in many mammals exposed to constant light. In a series of experiments simulating jet-lag conditions, melatonin infusions were found to alter the rate of re-entrainment of the circadian system after phase-shifting of the light cycle. These melatonin effects were influenced by light intensity and by phase of the hormone infusion. Measurements of melatonin receptor levels in the suprachiasmatic nuclei of Siberian hamsters has provided preliminary evidence for a daily rhythm of receptivity to melatonin in the circadian pacemaker of the mammalian hypothalamus, which may help explain the phase-dependency of melatonin effects on phase-shifting. Results of this study are relevant to the expanding clinical use of melatonin for treatment of a variety of temporal disorders, including jet-lag.

17. Document Analysis a. Descriptors

Jet-lag, melatonin, circadian, clock, sleep/wake cycles

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Approved for public release; distribution unlimited	unclassified	19
	20. Security Class (This Page) unclassified	22. Price

(See ANSI-Z39.18) See Instructions on Reverse OPTIONAL FORM 272 (4-77)

* GPO : 1993 0 - 281-526 (8393)

Figure A1
Report Documentation Page

Report # AFOSR - 91 - 0393

MELATONIN ACTION ON THE CIRCADIAN PACEMAKER IN SIBERIAN HAMSTERS

Janet M. Darrow, PhD
Department of Biology
414 Mugar Building
Northeastern University
Boston, MA 02115

October 31, 1992

Final Report for the Period 1 September 1991 - 31 August 1992

Prepared for:

LIFE SCIENCES DIRECTORATE
AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
BOLLING AIR FORCE BASE, D.C. 20332-6448

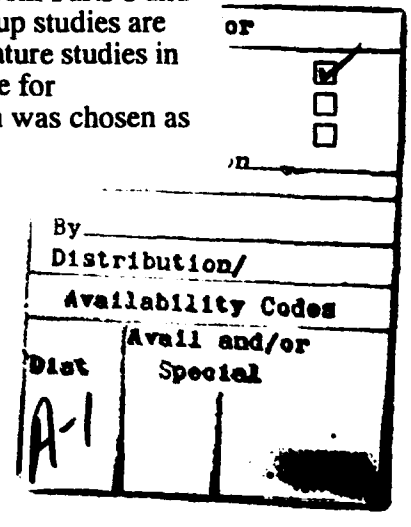
A) OBJECTIVES OF RESEARCH EFFORT:

The overall goal of this project was to characterize the interaction of melatonin with the circadian clock of mammals, using the melatonin-infused Siberian hamster as a model system. The significance of this project is to provide basic information relevant to the recently expanding clinical use of melatonin to treat temporal disorders (including jet-lag) as well as to ellucidate the function of specific melatonin receptors localized within the circadian clock (the suprachiasmatic nuclei, or SCN) of rodents and humans. The project has employed the following specific approaches:

- To determine if melatonin is an entraining agent ("Zeitgeber") for locomotor activity and body temperature rhythms in Siberian hamsters. This was to be ascertained under conditions of constant light (Part 1) and constant darkness (Part 2, of the original grant proposal).
- To determine if melatonin treatment protects the multioscillatory circadian system from "internal desynchronization" which occurs in numerous species after prolonged exposure to constant light (Part 1). Internal desynchronization is characterized by divergence of two or more rhythms (such as the wheel-running vs. temperature rhythms; or such as the "splitting" of morning vs. evening components of the wheel-running rhythm). If melatonin treatment affects the divergence of such rhythms, this suggests the hormone may affect strength of coupling among component oscillators of the circadian pacemaker system.
- To determine if melatonin treatment affects the rate of adjustment to phase-shifted light cycles (Part 3: the "jet lag experiments"). If so, then continued use of the Siberian hamster model system should be fruitful for characterizing melatonin treatment of jet lag and other temporal disorders in humans.
- To determine if there is a daily rhythm of receptivity to melatonin in the SCN by direct measurement of high affinity melatonin receptors at different times of day in the anterior hypothalamus and other brain sites of melatonin action (Part 4).

B. STATUS OF RESEARCH EFFORT

The majority of the experiments performed in the second year of grant were concerned with Parts 3 and 4 of the original proposal. Results of these studies are outlined below. The last experiment was completed on Sept 11, 1992. During the final months of project, malfunctioning of the hard drive on the Data Quest system hindered data print-out and analysis. Fortunately the majority of the data files were transferrable to MacIntosh SE30 and have been analyzed via Circadia software. Completion of the data analysis awaits retrieval of the remaining DataQuest files and replacement of the defective hard drive by the Northeastern University computer service. The majority of results from Parts 1 and 2 were described in the preceding annual report. Data from several follow-up studies are included in the above files yet to be analyzed. These included body temperature studies in adult male hamsters, the results of which appeared too variable to be useable for establishing phase or degree of splitting. Hence, the wheel-running rhythm was chosen as primary assay of phase for the second year studies.



Part 3. Melatonin infusions during phase-shifting of the light cycle : The Jet-Lag Experiments

GOAL: To determine if melatonin treatment affects the rate of adjustment to phase-shifted light cycles in Siberian hamsters. The very striking results of Experiment A, an intial study in this series, encouraged us to emphasize this line of inquiry.
METHODS: The basic experimental paradigm common to the majority of these experiments has been presented in diagrammatic form in the figures. In general, juvenile male hamsters were acclimated to wheel-running cages for several days, then anesthetized and implanted mid-dorsally with a subcutaneous cannula for daily rhythmic infusion of either melatonin (at 600 mg/day) or vehicle (saline). Two or three days later, the LD 16: 8 light cycle was phase-shifted, either delayed or advanced a specified number of hours, and the rhythmic infusions of melatonin or saline were continued for the next several days. In order to assess the phase of the circadian pacemaker system in response to phase-shifts and/or hormone treatment, the experimental room was released into constant darkness, and wheel-running rhythms which were being monitored throughout the study, were followed for an additional 5-7 days in the absence of daily infusions or the masking effects of the light/dark cycle. Circadian phase of locomotor activity onset on the first day of constant darkness (DD) was then extrapolated by linear regression from the DD free-running rhythm.

Experiment A) 8-Hour Phase Advance

Figure 1 presents the experimental paradigm and the daily wheel running activity pattern for a saline-infused and melatonin-infused hamster. Hormone treatment was given in two stages, before and after phase-shift of the light cycle, in order to test an experimental protocol which has been used in human jet lag studies (Arendt et al., 1987). Timing of the hormone treatment after the phase-shift was designed to simulate the nocturnal endogenous pineal melatonin pattern known for Siberian hamsters. (The experimental design used in this study is also presented in Figure 7 in a slightly different format.) The results obtained from this experiment were striking. Whereas all hamsters in the melatonin group phase-advanced in response to the phase shift, and rapidly attained proper phase relationship with the new light/dark cycle, saline-treated animals phase-delayed and showed atypical diurnal activity before release into DD. In Figure 2, activity onsets extrapolated from the DD free-run showed significant synchrony within the melatonin group, with mean onset approximating the time of lights-off (1100 hrs) in the new light/ dark cycle. In contrast, onsets for the saline group showed no significant clustering. These results suggested that melatonin might accelerate adjustment of the circadian system to phase-shifted light cycles, possibly through an action of the hormone directly on the circadian clock.

Experiment B) 8-hour phase delay, melatonin in new phase

The purpose of this study was to test whether melatonin affects the rate of phase shifting in response to a phase delay of the light cycle, if administered in phase with the night portion of the new light cycle. The experimental paradigm is diagrammatically outlined and explained in Figure 3. All hamsters responded to the phase-shifted light cycle by a phase delay of the wheel-running rhythm. However, the melatonin group adjusted to the new photoschedule more rapidly (Figure 4), with mean activity onset approaching the lights-off transition (0300 hrs) of the new light cycle within 4 days after the phase-shift. In contrast, mean onset for the saline group was significantly earlier ($p<.01$). These results suggest that melatonin treatment may affect the rate of re-entrainment to phase-delayed light cycles.

Experiment C) 8-hour phase advance, melatonin in old phase

Figure 5 describes a study designed to test if melatonin might alter the response to a phase-advanced light cycle, if infused during the night portion of the initial light cycle. No such effect was detected. Wheel-running activity records are shown in Figure 6 for a hamster from each treatment group, both of which advanced in response to the phase-shift. While all melatonin infused animals phase advanced, 6 of 8 control hamsters also advanced, 1 delayed and 1 showed activity too sparse for reliable determination of phase. No difference in mean activity onset values was observed between melatonin (10.214 $\pm$ 2.027 hrs) and saline treatment groups (8.084 + 0.65 hrs). The phase-advances observed in the saline group were unexpected, in light of the results of Experiment A, in which control animals consistently phase delayed in response to an 8 hour phase advance of the light cycle. Thus the subsequent experiment was performed to repeat Experiment A.

Experiment D) 8 hour phase advance, repeat of Experiment A

Figure 7 presents the experimental paradigm for this experiment. In this study, 6 of 8 saline-infused hamsters phase-advanced after the light cycle was shifted, as did all 10 of the melatonin-treated hamsters. If the two control animals which showed phase delays were deleted from calculations, no difference in mean activity onset values was discernable between the melatonin and saline groups.

Several aspects of the experimental conditions differed between Experiments A (performed at Wellesley College) and D (performed at Northeastern University), which might account for the disparity in results: e.g. the two animal rooms differed in general noise disturbance level and in uniformity of illumination. When light intensity was measured at the end of Experiment D, it was found to be at higher levels and more variable from cage to cage than when previously measured at the beginning of experiment C. Instead of a range of 10 to 20 lux measured inside the cages (the range of intensities used in Experiment A and for the beginning of Experiment C), light intensity ranged from 10 to 100 lux, depending on cage position on the rack. It was learned that fluorescent bulbs for the overhead lights had been replaced and that the position of the cage rack had been moved slightly in the room by maintenance personnel at some unknown time during these studies. Subsequent to this many additional precautions were taken to control lighting conditions.

Experiment E) Repeat of Experiment A at low light intensity

Because of indications that light intensity is a critical factor in these studies, the experimental paradigm shown in Figure 7 was repeated once again, at a lower range of light intensities (from 1 to 11 lux). The results are presented in Figures 8 and 9. In this study, the response of the saline-infused controls was similar to results of Experiment A; in that no phase advances were observed after the light cycle was shifted, and these hamsters showed atypical diurnal wheel-running activity prior to DD exposure. Of the 9 melatonin-treated hamsters, 5 showed phase advances and rapid adjustment to the new photoschedule within 4 days (see Figure 9 for example). Light intensity for this group ranged from 3 to 11 lux, (compared to the control group range from 1 to 9 lux). The 4 melatonin treated hamsters which phase delayed or showed little phase shifting response were exposed to less than 3 lux. Thus at the low light intensities tested in this study, melatonin apparently accelerates adjustment of some but not all hamsters to the phase-advanced light cycle, and this effect may depend on very small changes in light intensity.

Experiment F) 8 hour phase advance, non-infused hamsters

To characterize the threshold light intensity for phase shifting, 18 unoperated control hamsters were tested at a range of intensities from 2.5 to 44 lux, in the paradigm shown in Figure 10. Individual records are shown in Fig 11, for hamster C-2 (exposed to 25 lux) which phase advanced and adjusted rapidly, and for C-10 (exposed to 14 lux) which phase delayed and had not adjusted to the new light cycle even 10 days after the phase-shift. In Figure 12, it appears that light intensities above 16 lux result in phase advances, but below that level, yield a variable response.

Taken together with results of Experiment E, these data suggest a delicate interaction of melatonin and light intensity. One working hypothesis might be that light below 15 lux is insufficient to cause a phase shift, but that melatonin either increases sensitivity to light and/ or phase shifts the circadian system such that the system now responds. This synergism of melatonin and light is observed for the intensity range from 3 to 11 lux, but does not occur if light intensity is too low, e.g. below 3 lux. Whether or not the animal phase-advances or delays depends on the circadian phase at which light falls (relative to the break point of the phase response curve). Increased understanding of these results may emerge from further analysis of the activity records, re transients during phase-shifting, at least for some individuals where masking by the light /dark cycle was not obtrusive to determination of activity onsets.

Experiment G) 4-hour phase delay, evening melatonin

The next series of studies was designed to test the effectiveness of melatonin when given at a time of maximum sensitivity to the hormone. In rats, daily melatonin injections induce phase advances of wheel-running activity when administered in the evening (at or near CT10) but not at other times of the circadian day (Cassone et al., 1989). Preliminary evidence from collaborative studies with Dr. Cassone (Part 4 of this project), in which we have tested for a daily rhythm of melatonin binding in the SCN of Siberian hamsters, has suggested an afternoon elevation in melatonin receptor availability (Figure 20).

In Experiment G (Figure 13) , melatonin infusions were timed to overlap this interval of possible maximal sensitivity to the hormone, and to look for an effect of melatonin during a 4-hour delay of the light cycle. Light intensity was carefully controlled in the range of 3-5 lux for each animal. Locomotor activity records (Figure 14) showed a typical phase delay response from the saline-infused hamster, but only a slight phase delay in the melatonin-treated animal. Mean actitvity onsets (Figure 15) indicated that the saline group had achieved the proper phase relationship with the new photoschedule by 4 days after the phase shift, whereas the melatonin group had not. Thus melatonin treatment appeared to counteract or attenuate the phase-delaying effect of the light signal. This result would be consistent with a phase-advancing effect of melatonin, when administered near CT10.

Experiment H) 8-hour phase delay, evening melatonin

This experiment (Figure 16) was similar to the preceding one, except the light cycle was phase-delayed by 8 rather than 4 hours. The results in Fig 17 indicate a trend in the same direction, in that the control animals appeared to phase-delay more rapidly than the melatonin-treated hamsters, but the difference in mean onset values was not statistically significant . Thus a possible phase advancing effect by melatonin was less apparent when the system was challenged by a the larger phase-shift of the light cycle.

Experiment I) Light pulse vs. evening melatonin

In this experiment (Figure 18), the interaction of evening melatonin infusions with the phase-delaying effect of a 1-hour light pulse was tested. The timing of the light pulse (at 2200 hrs) was selected so as to bracket the maximal phase delay portion of the phase response curve in control animals. The results presented in Figure 19 demonstrate substantial phase delays for most saline-infused hamsters receiving light pulses between CT14 andCT18. In comparison, phase delays in the melatonin group were generally of lower amplitude. Furthermore, the timing of the light pulse for the melatonin-treated hamsters showed skewing to the right, in comparison to the saline controls. These results are consistent with a possible phase-advancing effect of melatonin and / or an attenuation of the phase-delaying effects of light. Further analysis of the data and additional data points are required to substantiate this possibility.

Part 4: Daily rhythm of receptivity to melatonin in the SCN

In collaboration with the laboratory of Dr. V. Cassone (Texas A&M University), we have obtained preliminary melatonin binding data for a portion of the 800 hamster brains which were collected at 2 hour intervals from pinealectomized and sham-pinealectomized Siberian hamsters. Figure 20 shows these preliminary results (obtained from in vitro incubation of brain slices with iodo-melatonin, in the presence or absence of nonradioactive melatonin) for the SCN and the pars tuberalis. In the SCN of pineal intact hamsters, specific melatonin binding was lowest during the night and elevated in late morning and afternoon. In pinealectomized hamsters, no diurnal pattern was evident. High background contributed to the variabilility in these results, as well as for the pars tuberalis. Therefore, these measurements will be repeated with iodo-melatonin of higher specific activity.

C. MANUSCRIPTS IN PREPARATION

Darrow J.M., Doyle S.E. and Goldman, B.D. Effects of daily melatonin infusions on the circadian clock and reproductive system of Siberian hamsters exposed to constant light.

Darrow, J.M. and Doyle, S.E. Effects of daily melatonin infusions on locomotor activity rhythms of Siberian hamsters exposed to constant darkness.

Darrow, J.M. and Doyle, S.E. Melatonin infusions alter entrainment of Siberian hamsters to phase-shifted light cycles.

Darrow, J.M., S.E. Doyle and V. Cassone, Diurnal pattern of melatonin receptor availability in the SCN and pars tuberalis of pinealectomized and pineal-intact Siberian hamsters.

D. PROFESSIONAL PERSONNEL

Susan E. Doyle, Wellesley College Honors graduate

E. INTERACTIONS

DATA PRESENTATION:

Darrow, J.M. and S. E. Doyle, Melatonin infusions in wheel-running Siberian hamsters in constant darkness: Possible entrainment of the locomotor activity rhythm. Annual Meeting Soc. Research Biological Rhythms, Jacksonville, Fla, 1990.

Darrow, J.M. Invited seminar speaker at Northeastern University, Vassar College, Wellesley College, Williams College, 1990-91.

Darrow, J.M. and S.E. Doyle, Melatonin affects rate of adjustment to phase-shifted light cycles in Siberian hamsters. Annual Meeting Soc. Research Biological Rhythms, Jacksonville, Fla, 1992.

COLLABORATIONS:

With Dr. Vincent Cassone, Texas A &M University
With Dr. Fred C. Davis, Northeastern University

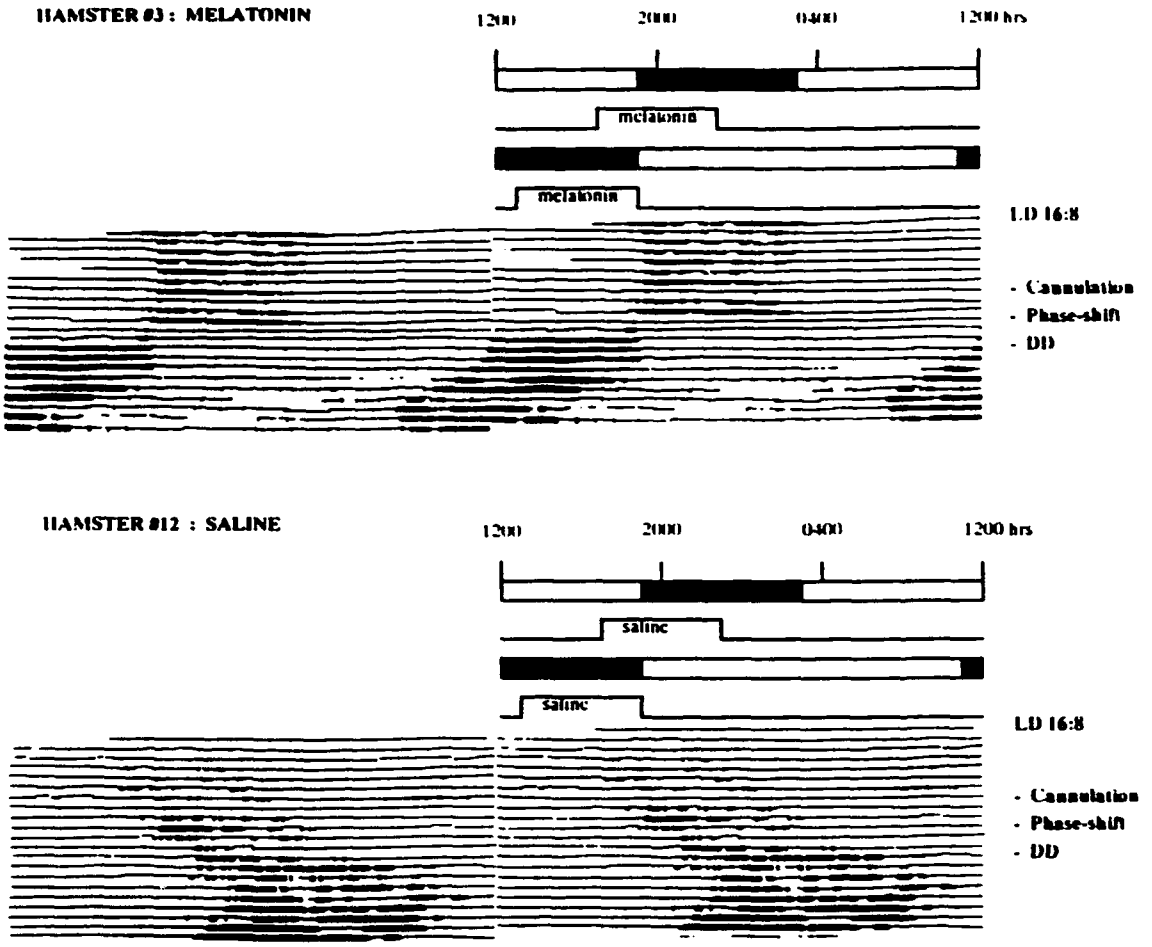


FIGURE 1

Double-plotted wheel running activity records from Experiment A, for two individual male hamsters, receiving daily saline or melatonin infusions (6 hours per day; 600 ng melatonin/day). Infusions began on the day of cannulation surgery, at the time indicated by the upper square wave above the figure. Three days later, the light cycle was phase-advanced by 8 hours (as indicated by the horizontal light and shaded bars above the activity record) and the infusion interval was phase-advanced as indicated. Four days later, the infusion pumps were turned off and the room was released into constant darkness (DD) at the lights-off transition, in order to monitor the free-running locomotor activity rhythm for the subsequent 7 days.

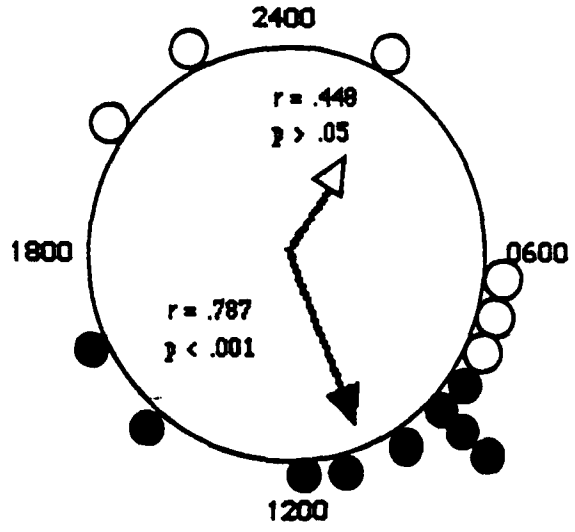


FIGURE 2

Phase of extrapolated DD onsets from Experiment A, plotted on a circular 24-hour time scale, for individuals treated with melatonin (closed circles) or saline (open circles). Each data point is the intercept on day 1 of DD, obtained from the linear regression analysis of the DD free-run. The direction of the arrow denotes mean phase of onsets. The length of the solid arrow (r) indicates significant clustering of phases within the melatonin group ($p<.001$, as determined by the Raleigh test), in contrast to the scattered distribution of the saline-treated hamsters.

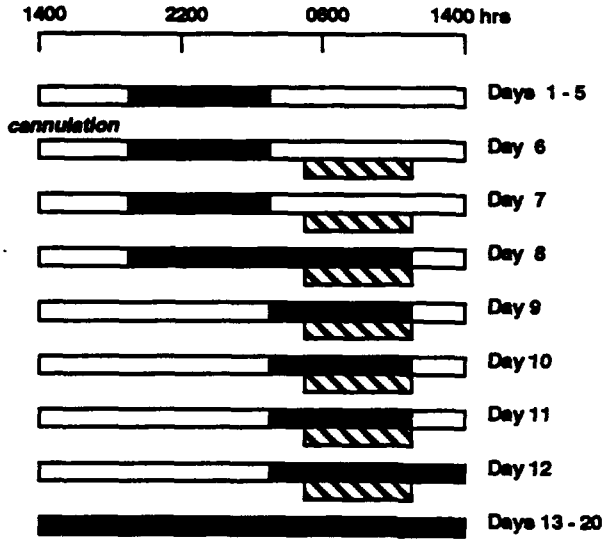


FIGURE 3

Experimental paradigm used for Experiment B, testing melatonin effects on an 8-hour phase delay of the light cycle. The light vs. dark portions of the daily light cycle are indicated by open vs. solid segments of the long horizontal bars. Hatched horizontal bars indicate phase of the 6-hour melatonin or saline infusions, programmed to anticipate and then coincide with the night portion of the phase-shifted light cycle. Juvenile male hamsters raised from birth on LD16:8 were transferred to wheel-running cages for 5 days, then subcutaneously cannulated. After two days of infusions, the light cycle was phase-delayed by 8 hours, and infusions continued until Day 12. Hamsters were then released into constant darkness for 7 days to assess phase of the locomotor activity rhythm.

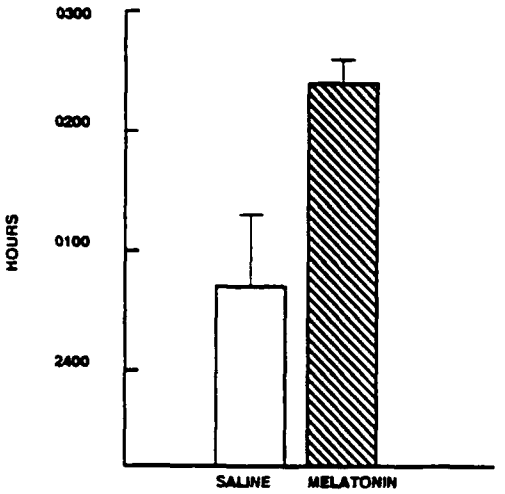


FIGURE 4

Phase of locomotor activity onsets for saline and melatonin infused hamsters in Experiment B, measured on Day 1 of constant darkness. The mean value (+S.E.M.) for the melatonin treatment group (02.375$\pm$.206 hrs) differed significantly ($p<.01$) from that of the saline group (00.713$\pm$.562 hrs).

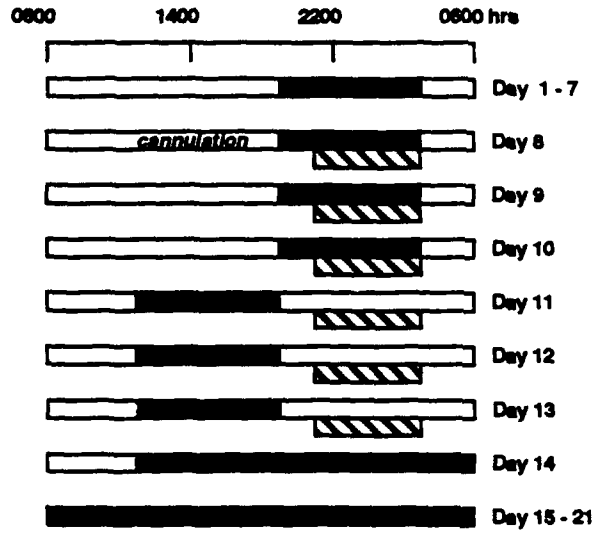
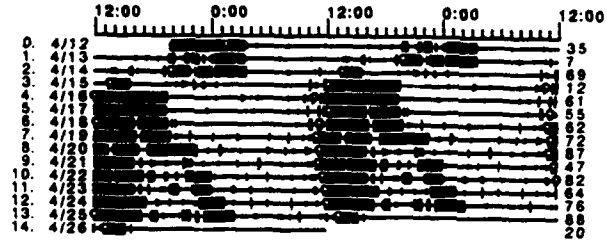


FIGURE 5

Protocol for Experiment C, testing an 8-hour phase advance of the light cycle, with melatonin or saline infusions set to coincide with the night portion of the initial light/dark cycle. Symbols used are explained in the legend to Figure 3.

HAMSTER C-3: SALINE



HAMSTER C-5: MELATONIN

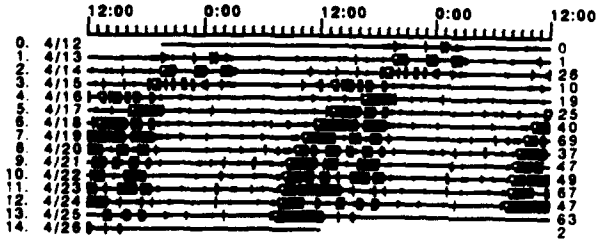


FIGURE 6

Double-plotted wheel-running activity records from Experiment C, beginning on the day of cannulation, for individual hamsters receiving melatonin or saline. Open circles designating each day's activity onset were determined by the *Circadia* data analysis program.

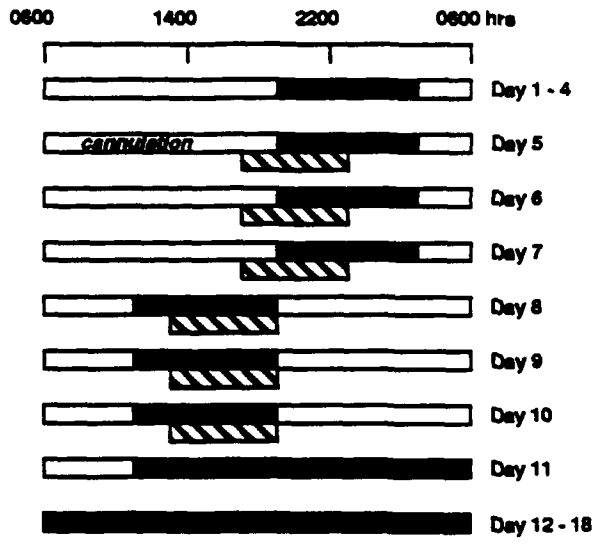


FIGURE 7

Protocol for Experiments D and E, testing an 8-hour phase advance of the light cycle, and a sequential advance of the melatonin or saline infusion times. These two experiments differed only in the light intensity used (see text).

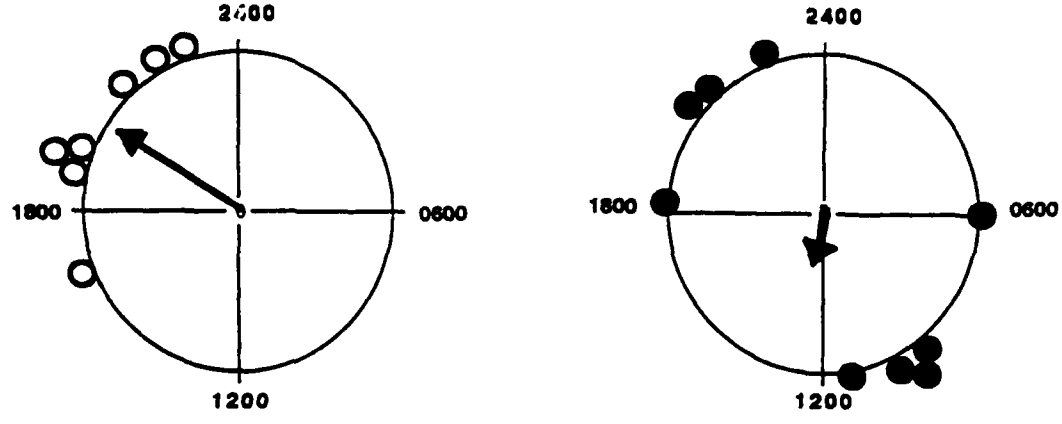
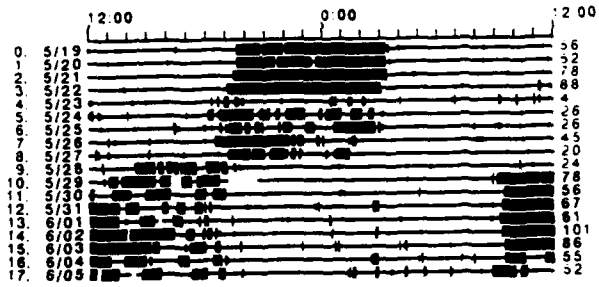


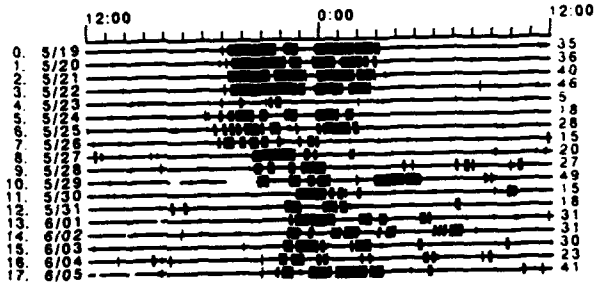
FIGURE 8

Phase of extrapolated DD onsets from Experiment E plotted on circular time scales for hamsters treated with melatonin (closed circles, n=9) or saline (open circles, n =7). Each data point is the intercept on the first day of DD, obtained from linear regression analysis of the DD free-run. The length of the arrow for the saline infused hamsters (r = 0.87) indicates significant synchronization within this group (p <.01, as determined by the Raleigh test); with a mean phase of 20.05 hours. In contrast, clustering of the phase of activity onsets was not observed for the melatonin treatment group (r = .146).

HAMSTER C-5: MELATONIN



HAMSTER C-10: MELATONIN



HAMSTER C-3: SALINE

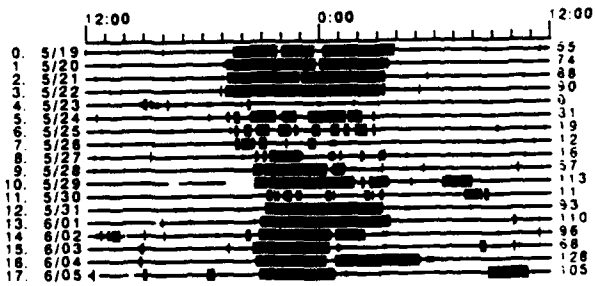


FIGURE 9

Wheel-running activity records for three individual hamsters of Experiment E. Cannulation surgery was on 5/23, Day 5 of the experimental paradigm in Fig. 7.

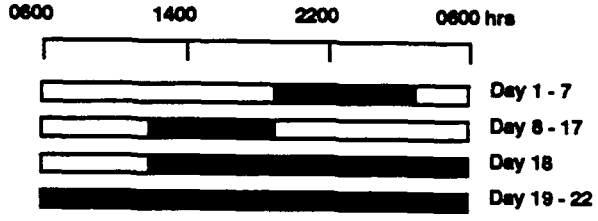
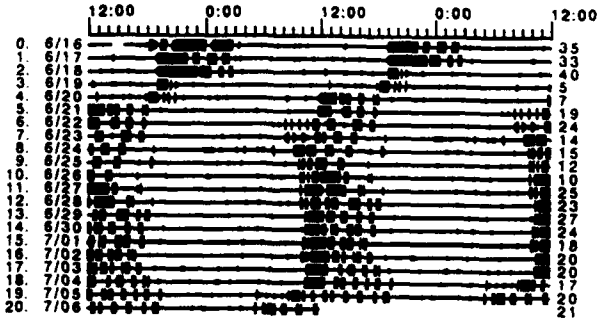


FIGURE 10

Protocol for Experiment F, testing the response of unoperated hamsters to an 8-hour phase advance of the light cycle. In this experiment, light intensity was purposely varied from one animal to the next, such that a range of intensities, from 2.5 to 44 lux was tested.

HAMSTER C-2



HAMSTER C-10

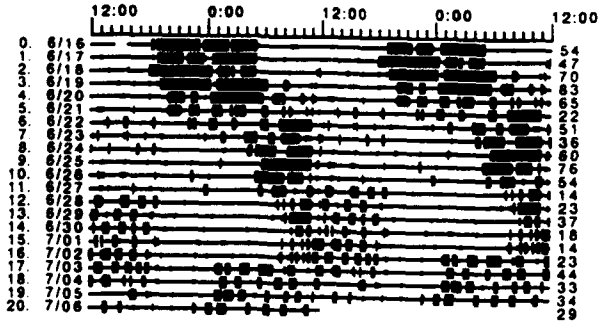


FIGURE 11

Double-plotted wheel-running activity records for two hamsters from Experiment F, one showing an advance, the other a delay in response to the shifted light cycle.

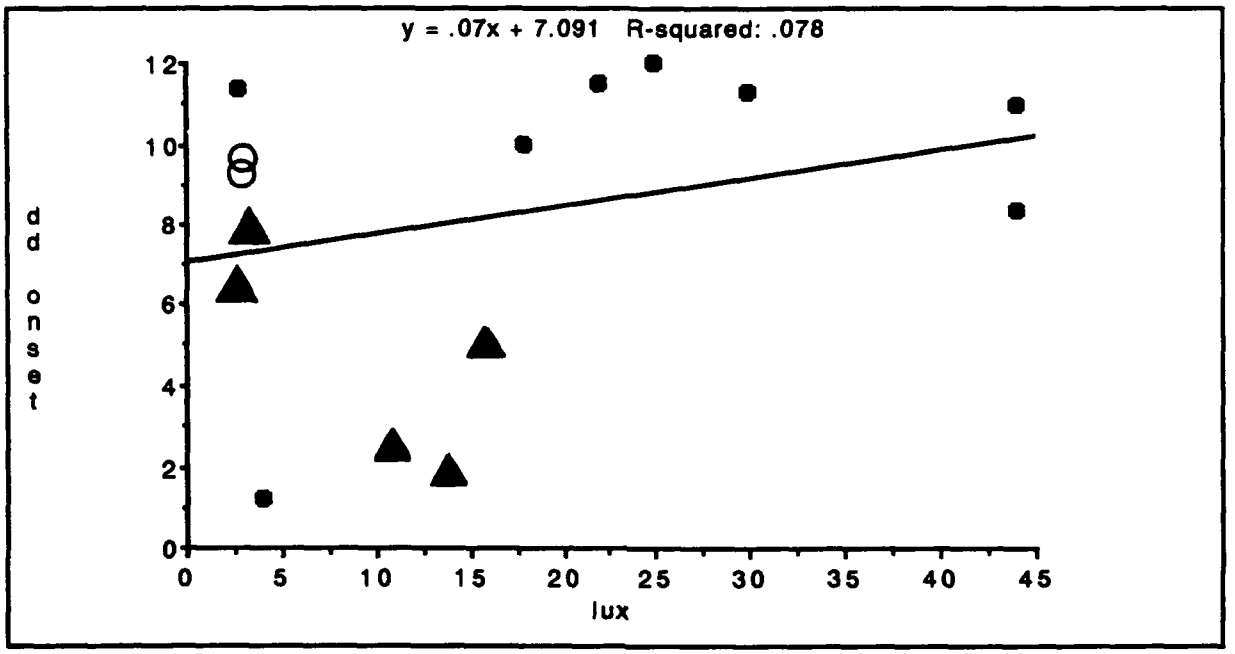


FIGURE 12

Phase of activity onset from individual hamsters in Experiment F plotted as a function of light intensity measured within each animal's cage. Small solid circles denote hamsters which phase-advanced in response to the phase-shifted light cycle, solid triangles those which phase delayed, and open circles those which showed splitting of wheel-running activity.

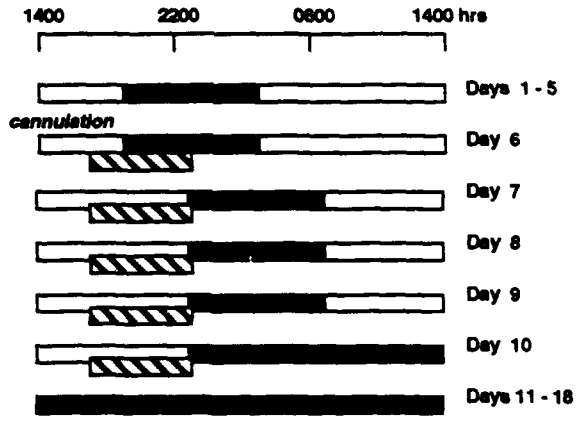


FIGURE 13

Protocol for Experiment G, testing a 4-hour phase delay of the light cycle. The 6-hour melatonin and saline infusions were programmed to precede the lights-off transition for the shifted light cycle. Light intensity was carefully set at 3-5 lux within each cage for this and the two subsequent experiments.

HAMSTER C-4: SALINE

HAMSTER C-17: MELATONIN

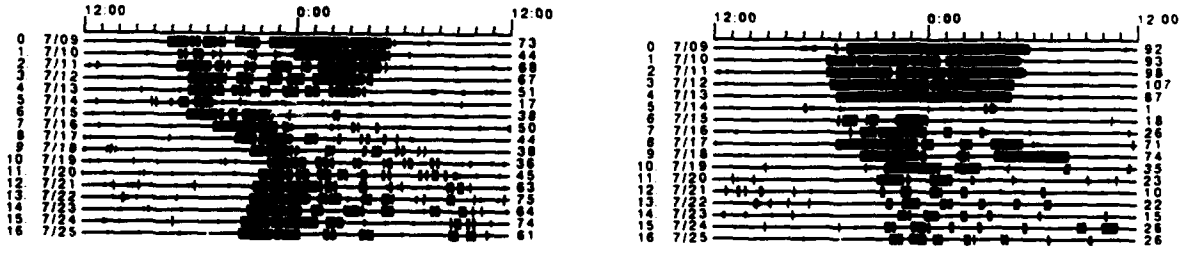


FIGURE 14

Wheel-running activity records from Experiment G for a melatonin- and a saline-infused hamster .

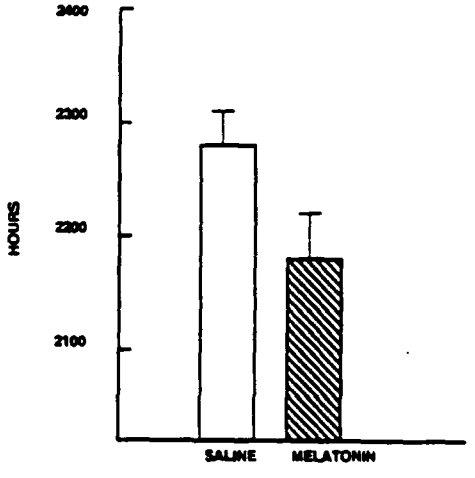


FIGURE 15

Phase of locomotor activity onset for hamsters of Experiment G, measured on day 1 of constant darkness. The mean value for saline-infused hamsters was significantly later ($p<.05$) than for melatonin-treated animals.

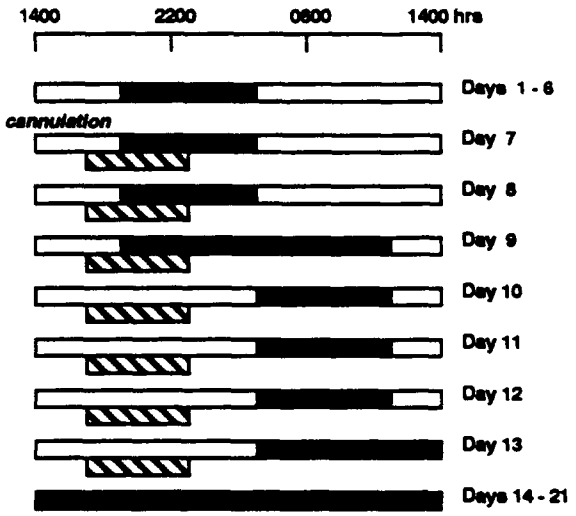


FIGURE 16

Protocol for Experiment H, testing an 8-hour phase delay of the light cycle, with infusions beginning at the same time of day as in Experiment G.

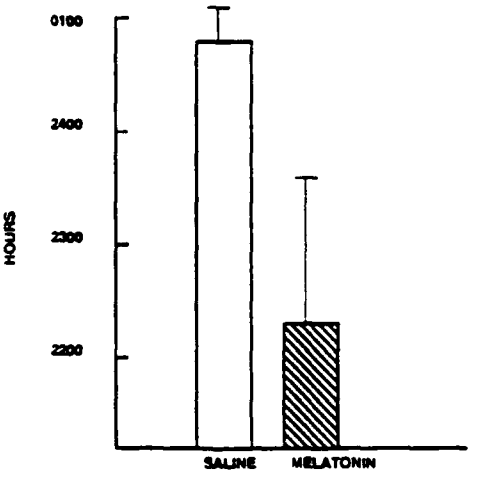


FIGURE 17

Phase of locomotor activity onset from hamsters of Experiment H, measured on day 1 of constant darkness. The mean value for the saline-infused hamsters was later than that for the melatonin group, but this difference was not statistically significant.

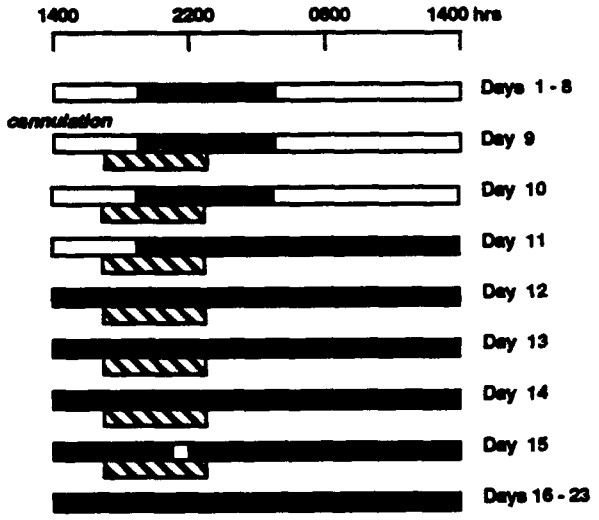


FIGURE 18

Protocol for Experiment I, testing the effect of melatonin on the hamster's response to a 1-hr light pulse. Beginning on Day 9, hamsters received 6-hour infusions of melatonin or saline at the time indicated, then on Day 11, were released into constant darkness. On Day 15, room lights were turned on for 1 hour, (denoted by the open square) at the same low intensity (3-5 lux) as used throughout Experiments G, H and I. DD exposure continued for the subsequent 7 days, to obtain the post-pulse phase of activity onset.

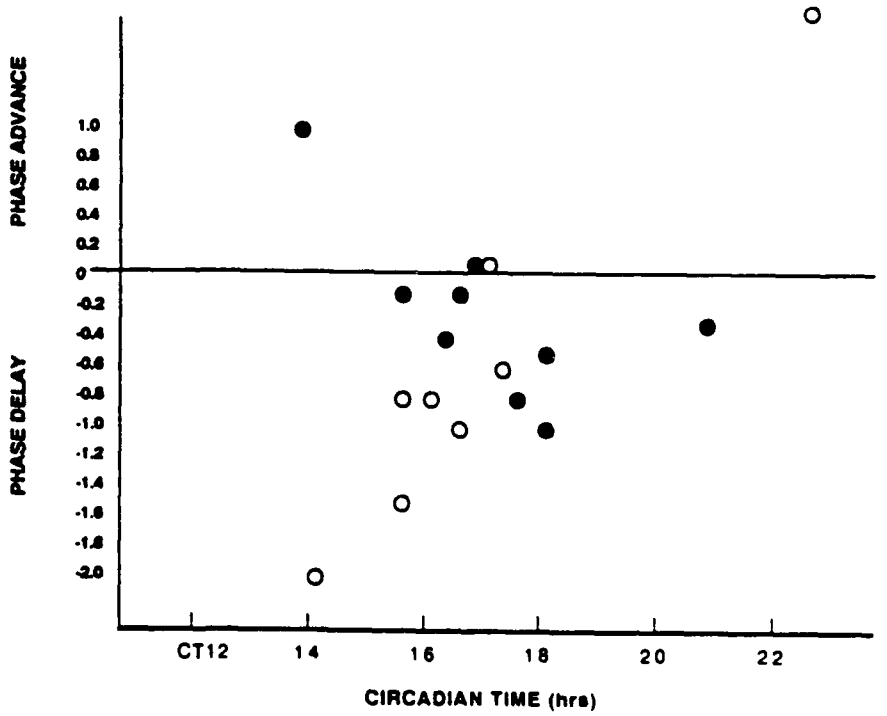


FIGURE 19

Phase-shifting effect of the 1-hour light pulse in Experiment I for melatonin-infused (closed circles) and saline-infused hamsters (open circles). Circadian time at which the light pulse occurred was determined for each hamster relative to its activity onset on the day of the pulse. Magnitude of the phase advance or phase delay was calculated from each individual's post-pulse DD free-run, and is expressed (in hours) along the y-axis.

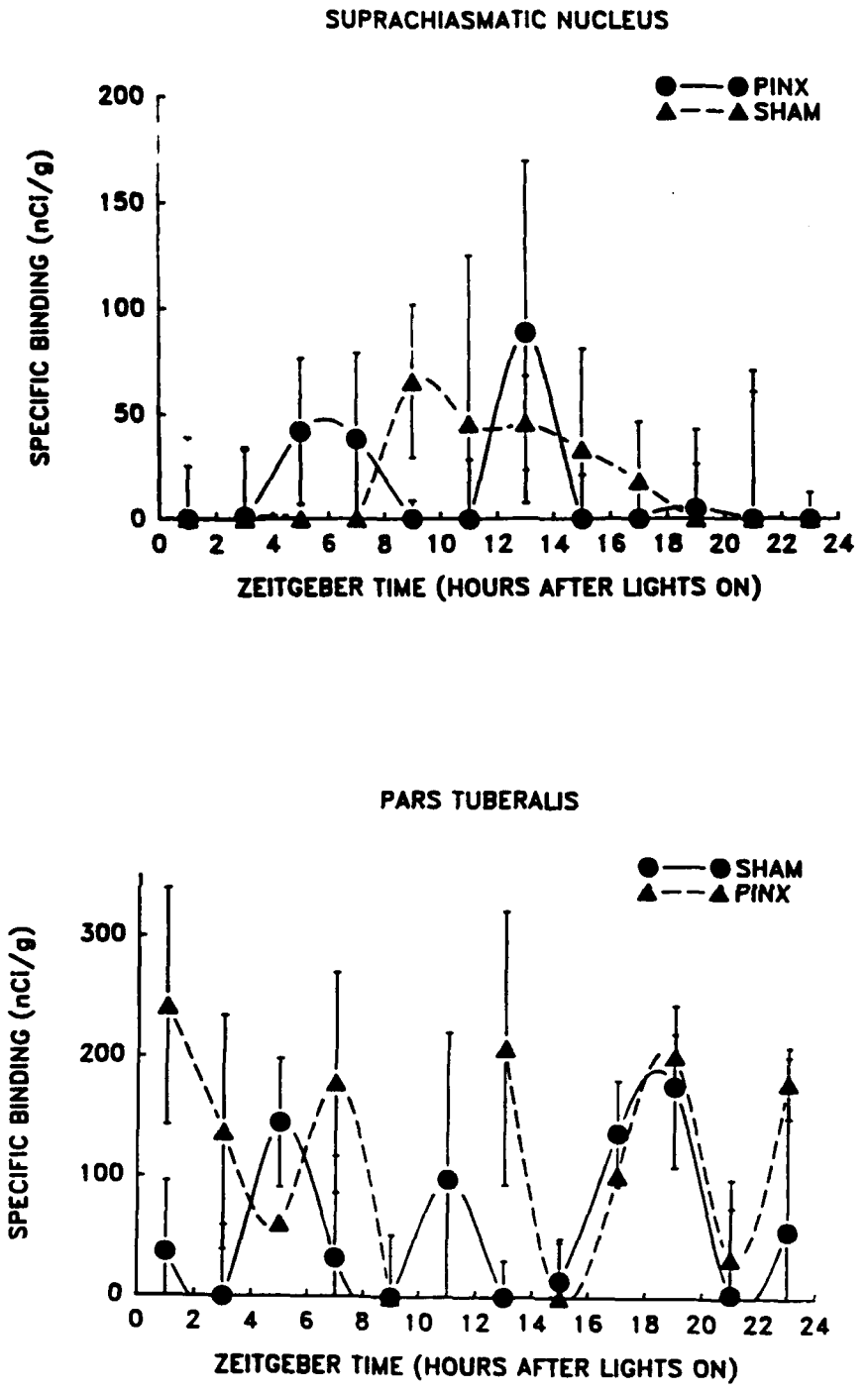


FIGURE 20

Daily pattern of melatonin binding in the SCN (upper panel) and pars tuberalis (lower panel) measured at 2-hour intervals in pinealectomized and sham-operated Siberian hamsters. 8 to 12 animals were sampled per time point, on an LD 16:8 light cycle.